LEAD STUDIES.

VIII. THE MICROCHEMICAL DETECTION OF LEAD.

By LAWRENCE T. FAIRHALL.

(*From the Laboratories of Physiology, Harvard Medical School, Boston.*)

(Received for publication, July 2, 1923.)

From the view-point of the clinical laboratory, the accurate determination of lead in biological material is a relatively complicated procedure. The several factors which account for this have already been discussed (1). A relatively simple qualitative test for lead would therefore accomplish two things: (*a*) It could be used to confirm the quantitative determination of the small amounts used; and (*b*) it could be readily used in the clinic where only qualitative results are usually desired. It was for these reasons that distinctive microchemical methods were studied.

The so called "triple nitrite test" of Behrens and Kley (2) is perhaps the most characteristic of the microchemical tests for lead. It depends upon the formation of a hexanitrite of potassium, copper, and lead—$K_2CuPb(NO_2)_6$. This hexanitrite is readily identified under the microscope by its regular crystalline structure. For this reason the test is extremely delicate and far surpasses the usual microanalytical tests for lead, such as the iodide and chromate tests. The iodide test is not particularly distinctive under the microscope, nor is it wholly satisfactory because of the relative solubility of lead iodide. Lead chromate, while very insoluble, is invariably precipitated in an amorphous condition so that identification is difficult.

The triple nitrite test consists in adding small crystals, successively, of copper acetate, sodium acetate, and potassium nitrite to a drop of the lead solution which had been slightly acidified with acetic acid. Sodium acetate is added in order to buffer free mineral acids, which interfere with the test. The hexanitrite crystallizes in regular rectangular plates or cubes (Figs. 1 and 2),

This is an Open Access article under the CC BY license.

usually black, but occasionally dark red when the plates are thin. With relatively large amounts of pure lead salt the test seldom fails when made in this way. Frequent failures have been obtained, however, when this test was applied to traces of lead precipitated in the analysis of biological material. Here it frequently happens that the lead is present in such small amounts that considerable analytical skill is necessary to make an exact estimation. A study was therefore made for the purpose of applying this test to clinical material and for defining more exactly the conditions under which the hexanitrite would crystallize in low concentrations.

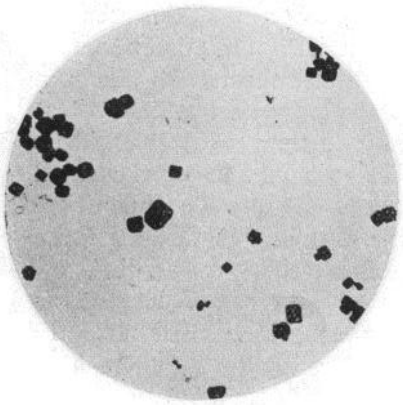

FIG. 1.

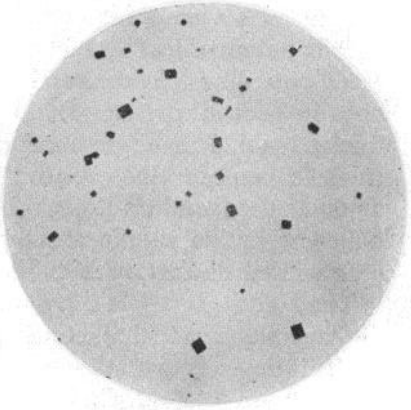

FIG. 2.

FIG. 1. Showing the regular rectangular plates or cubes of hexanitrite crystals. × 85.

FIG. 2. Showing the regular rectangular plates or cubes of hexanitrite crystals. × 350.

In order to insure constant results with this test it was found that definite quantities of reagent must be used; the procedure as usually outlined is altogether too indefinite. Instead of adding crystals of the solid salts, therefore, the reagents in solution form should be measured from capillary pipettes. The latter may be made readily by drawing out a piece of 4 mm. glass tubing to a capillary and blowing a bulb at one end. The stem may be calibrated roughly by marking two points, representing a volume of 5 c. mm. (about 1/10 of a normal drop). Using measured quanti-

ties of reagents of definite concentration results not only in reproducible results but also avoids any tendency to "drown" the preparation—a frequent cause of failure noted by Schoorl (3). Solutions of the following composition are required: Sodium acetate 4 per cent, pure copper acetate 2 per cent, and 10 per cent acetic acid.

The purity of the lead solution to be tested is a decided factor in the success of the test. The lead must be separated in such a way therefore that contamination is reduced to a minimum. Ammonium, iron, and calcium salts interfere with the test, even when a relatively large amount of lead is present. The most convenient method of separating the lead is as the sulfide. This is possible even with a very minute amount of lead in the presence of a relatively great concentration of other inorganic salts. The means of accomplishing this, however, must be a refinement of the ordinary analytical procedure. The acidity of the solution must be reduced as far as possible in order to precipitate the maximum amount of lead; for this reason the solution should be made just acid to methyl orange by the least amount of hydrochloric acid possible. The physical separation of the colloidal lead sulfide presents another difficulty. 0.005 mg. of lead in 15 to 20 cc. of solution gives a barely visible yellow color on saturation with hydrogen sulfide. Centrifugation alone is insufficient to throw out the lead sulfide in such a case. A convenient means of accomplishing this, however, is to add first of all a drop of the 2 per cent copper acetate solution and 1 cc. of a saturated solution of ammonium sulfate. The ammonium sulfate serves to coagulate the colloidal sulfides and the copper sulfide on centrifugation carries down the lead sulfide by *entrainment*.

Since traces of inorganic salts interfere seriously with the test, these must be removed by careful washing. This is readily accomplished by decantation, as the sulfide packs well in the bottom of the centrifuge tube. If the washing is carefully done it is usually unnecessary to reprecipitate the lead as sulfide.

Cesium or thallium salts improve the sensitiveness of the test for lead, as the corresponding hexanitrites are much more insoluble than the potassium hexanitrite. With thallium the sensitiveness is particularly increased. However, the crystals of the thallium compound are very minute and much more difficult to identify than

the potassium salt. Also crystals of thallous chloride, which occasionally form, are apt to cause confusion. For the clinical detection of lead, the more common potassium nitrite is quite sufficiently sensitive as a reagent.

Procedure.

In testing for lead in organic material, it is essential to oxidize the organic substances present before lead will respond to the ordinary precipitation reactions. The material should be baked and ashed and the ash completely dissolved in hydrochloric acid.[1] The volume of solution should be about 25 cc. After neutralizing the solution thus secured it should be made just acid to methyl orange with a small amount of dilute hydrochloric acid. 1 cc. of a saturated solution of ammonium sulfate and 1 drop of 2 per cent copper acetate solution are added and the cold solution is saturated with hydrogen sulfide gas. The sulfides are thrown out by centrifugation and washed at least three times by decantation, the water being drained completely from the precipitate each time by means of a capillary tube. The washing must be thorough and the wash water completely drained from the sulfide precipitate each time, as small amounts of inorganic salts seriously interfere with the test. The centrifuge tube containing the sulfides, drained free from water, is then placed in a beaker of boiling water and 2 drops of nitric acid are added. Some of the solution thus obtained should be drawn up into a capillary tube and a drop or so evaporated to dryness on a microscope slide. 5 to 10 c. mm. of 4 per cent sodium acetate solution should then be added and the residue completely dissolved. In case copper acetate was not added previously to aid in the precipitation of the lead sulfide, it is necessary also to add 5 c.mm. of the 2 per cent copper acetate solution. The residue must be thoroughly mixed with these so that all particles dissolve. The material should be collected into 1 droplet and again evaporated to dryness. When carefully done the residue will consist of a rim about 4 mm. in diameter in which the greater part of the salts are concentrated. The slide should then be chilled on ice, 5 c.mm. of 10 per cent acetic acid and a small crystal of potassium nitrite are added. This nitrite solution should

[1] Fairhall (1), p. 14.

be centrally placed in the dry residue and allowed to diffuse to the outer edge. This slow diffusion permits the hexanitrite crystals to form at various places under optimum conditions of concentration. The small black cubes and squares are usually found in the outer rim scattered among the crystals of copper acetate. Instead of adding a small crystal or two of potassium nitrite to the acetic acid droplet, 5 c.mm. of a 20 per cent potassium nitrite solution acidified with acetic acid may be added directly. The former method is the more convenient, however, since the acid-nitrite solution rapidly decomposes. An excess of potassium nitrite serves to reduce the solubility of the hexanitrite, though this is opposed to the statement of Chamot (4) that the hexanitrite is soluble in an excess of the salt. That this is not so may be shown by adding solid potassium nitrite to a saturated solution of the hexanitrite. This produces an immediate precipitate of the black crystals showing that an excess diminishes the solubility instead of increasing it. Therefore, a slight excess of potassium nitrite is an advantage. However, an excess of acetic acid solution is to be avoided, as the crystals are rather soluble in water. The amount of acetic acid is restricted for this reason to 5 c.mm.

By evaporating a drop of a very dilute solution of a pure lead salt on a microscope slide it is possible to estimate the sensitiveness of limit of this test for lead. This sensitiveness is given as 0.03μgm. by Behrens and Kley[2] and 0.003μgm. by Emich (5). It is quite out of the question, however, to expect to separate any such small amount of lead from several grams of ash and to identify it. Indeed, 2 or 3μ gm. of lead salt dissolved in 15 to 20 cc. of water fail to show the slightest color change with hydrogen sulfide. With the above procedure, however, experiments repeatedly have shown that 0.001 mg. or 1μ gm. of lead may be separated and readily identified from 15 cc. of a solution containing other salts.

Effect of Bismuth.

A great many of the reactions of bismuth are so similar to those of lead that it is somewhat difficult to identify either metal when present in amounts less than 1 mg. Since bismuth salts are occasionally administered medicinally in cases of lead poisoning,

[2] Behrens and Kley (2), p. 65.

the distinction between the two must be closely drawn. When bismuth is present a white precipitate is produced by sodium acetate. This precipitate obscures or prevents the formation of the hexanitrite. The greater part of the bismuth may be separated, however, by converting it into the insoluble basic nitrate by dilution with water. The most satisfactory method is to evaporate the nitric acid solution of nitrates in the centrifuge tube to dryness and then add 1 or 2 drops of water. A white precipitate of the bismuth oxynitrate forms, while the lead nitrate passes into solution. A drop of this solution may be filtered readily in a modified Savage filtration tube (6). For this purpose a fine capil-

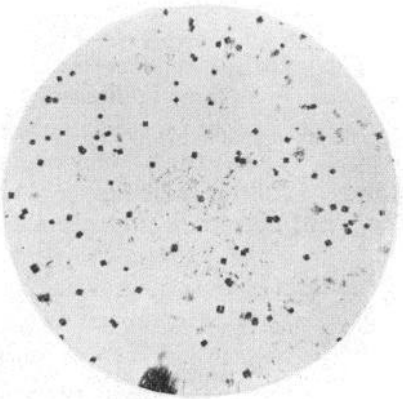

FIG. 3.

FIG. 3. Showing the hexanitrite crystals formed in the presence of a small amount of bismuth. × 350.

lary tube having a tiny plug of absorbent cotton at one end is dropped into the solution. The clear liquid is drawn into the tube by capillary action and, after breaking off that part of the tube containing the plug, may be transferred to the slide. The small amount of bismuth left in solution no longer interferes with the test for lead. Fig. 3 is a photomicrograph of such a test where the ratio of bismuth to lead was originally 100:1. The very slight amount of bismuth still present separates as an amorphous precipitate, but does not prevent the formation of the hexanitrite crystals.

Purification of Reagents.

Most of the reagents employed for the separation and identification of lead will be found to contain that metal in minute amounts. Purification of these reagents is therefore a necessary first step in the application of this test for lead. Fortunately this is a simple matter in most cases. With ammonium sulfate, for example, the cold solution may be saturated with hydrogen sulfide gas, allowed to stand over night, the solution filtered from the slight precipitate of sulfide, and the excess hydrogen sulfide removed by boiling. Potassium nitrite often contains a relatively large amount of lead. A very pure grade of potassium nitrite may be prepared from silver nitrite. In order to do this, silver nitrate should be added to the filtered solution of potassium nitrite and the resulting precipitate of silver nitrite washed well with cold water. This silver nitrite is then dissolved in boiling water and an equivalent amount of pure potassium chloride added. The pure potassium nitrite in solution may then by crystallized by evaporating this solution to a small bulk. Acids and ammonia may be purified by the method devised by Lenz (7).

CONCLUSION.

A modified procedure for the hexanitrite test for lead has been described and applied to the detection of lead in biological material. By means of this method as small a quantity as 1μ gm. of lead may be separated and identified in the presence of a considerable amount of other inorganic salts. The test may be used as a satisfactory and relatively simple clinical method for the detection of lead.

BIBLIOGRAPHY.

1. Fairhall, L. T., Lead studies. 1. The estimation of minute amounts of lead in biological material, *J. Ind. Hyg.*, 1922, iv, 9.
2. Behrens, H., and Kley, P. D. C., Mikrochemische analyse, Leipsic, 3rd edition, 1915, 28.
3. Schoorl, N., Beiträge zur mikrochemischen analyse. II. Analyse der Silbergruppe, *Z. anal. Chem.*, 1908, xlvii, 216.
4. Chamot, E. M., Elementary chemical microscopy, New York, 1916, 328.
5. Emich, F., Lehrbuch der mikrochemie, Wiesbaden, 1911, 68.
6. Savage, G. H., A filter for microchemical analysis, *J. Appl. Micros.*, 1900, iii, 678.
7. Lenz, W., Mikrochemische Reagenzien, *Z. anal. Chem.*, 1913, lii, 90.